UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

October 8,. 2002

MEMORANDUM

SUBJECT: Pyraflufen-Ethyl - Report of the Cancer Assessment Review Committee

FROM: Jessica Kidwell Jessica Kidwell
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Ghazi Dannan, Toxicologist
Registration Action Branch 3
Health Effects Division (7509C)

And

Mary Rust, Risk Assessor
Registration Action Branch 3
Health Effects Division (7509C)

The Cancer Assessment Review Committee met on August 14, 2002 to evaluate the carcinogenic potential of Pyraflufen-Ethyl. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

TXR #0050930

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF **PYRAFLUFEN-ETHYL** **PC CODE 030090**

FINAL REPORT

October 8, 2002

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION — Ghazi Dannan, Toxicologist

DOCUMENT PREPARATION — Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam	
Karl Baetcke	
Marion Copley	
Kerry Dearfield	
Virginia Dobozy	
Nancy McCarroll	Absent
Tim McMahon	
Esther Rinde	
Joycelyn Stewart	
K. Clark Swentzel	
Linda Taylor	

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher, Pathology Consultant	Absent
Lori Brunsman, Statistical Analysis	

OTHER ATTENDEES: Stephen Dapson, HED/RAB3, Leung Cheng, HED/RAB3

TABLE OF CONTENTS

EXECUTIVE SUMMARY 1

I. INTRODUCTION 3

II. BACKGROUND INFORMATION 3

III. EVALUATION OF CARCINOGENICITY STUDIES 4
1. Combined Chronic Toxicity and Carcinogenicity Study in CR:CD Rats 4
2. Carcinogenicity Study in (SPF) ICR Crj:CD-1 Mice 11

IV. TOXICOLOGY 21
1. Metabolism 21
2. Mutagenicity 22
3. Structure-Activity Relationship 23
4. Subchronic, and Chronic Toxicity 24
5. Mode of Action (MOA) Studies 27

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE 31

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL 32

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL 33

VIII. BIBLIOGRAPHY 34

EXECUTIVE SUMMARY

On August 14, 2002 the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of pyraflufen-ethyl. This was the initial assessment of this chemical for carcinogenicity by the HED CARC.

Dr. Ghazi Dannan of the Registration Action Branch 3 presented the chronic toxicity/carcinogenicity studies in CR:CD rats and ICR (Crj:CD-1) mice by describing the experimental design, reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested, and presenting the weight of the evidence for the carcinogenicity of pyraflufen-ethyl. Dr. Dannan also discussed the toxicology, metabolism, mechanism of action and mutagenicity studies, as well as structure-activity relationships.

Pyraflufen-ethyl was administered in the diet to groups of 70 CR:CD rats/sex/dose at dose levels of 0, 80, 400, 2000, and 10,000 ppm (3.4, 17.2, 86.7, and 468.1 mg/kg/day for males and 0, 4.4, 21.8, 111.5, and 578.5 mg/kg/day for females) for 104 weeks in a chronic toxicity/carcinogenicity study; and to 60 (SPF) ICR (Crj:CD-1) mice/sex/dose at dose levels of 0, 200, 1000, or 5000 ppm (0, 20.99, 109.7, and 546.8 mg/kg bw/day for males and 0, 19.58, 98.3, and 523.7 mg/kg bw/day for females) for 78 weeks in a carcinogenicity study.

The CARC concluded that pyraflufen-ethyl showed evidence of carcinogenicity based on the following:

- Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 5000 ppm dose group with the controls, for hepatocellular adenomas, and combined adenomas, carcinomas and/or hepatoblastomas, all at $p< 0.01$. There were also significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for hepatocellular adenomas, and combined adenomas, carcinomas and/or hepatoblastomas, both at $p< 0.05$. The increased incidences of hepatocellular adenomas at 1000 and 5000 ppm were robust and exceeded the historical control mean and range. The CARC considered the increase in hepatocellualar adenomas and combined adenomas, carcinomas, and/or hepatoblastomas at 1000 and 5000 ppm to be treatment-related in males. There was no treatment-related increase in hepatocellular carcinomas.

- Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 5000 ppm dose group with the controls, for hepatocellular adenomas, and combined adenomas and/or carcinomas, all at $p< 0.01$. The increased incidence of hepatocellular adenomas at the high dose (5000 ppm) was robust and exceeded the historical control mean and range. The CARC considered the increase in hepatocellular

adenomas and combined adenomas and/or carcinomas to be treatment-related in females. There was no treatment-related increase in hepatocellular carcinomas.

- The highest dose tested of 5000 ppm was considered to be adequate, but not excessive, in both sexes, to assess the carcinogenicity of pyraflufen-ethyl in ICR (Crj:CD-1) mice. This was based on liver toxicity (centrilobular hepatocellular swelling in both sexes, brown pigment deposition in the Kupffer cells) and anemia, albeit slight, at 5000 ppm. Additional support derive from the results of the 28-day feeding study in which liver toxicity and anemia were observed at 3000 and 10,000 ppm.

- There was no treatment-related increase in any tumors in male and female rats.

- The highest dose tested of 10,000 ppm was considered to be adequate, but not excessive, in both sexes, to assess the carcinogenicity of pyraflufen-ethyl in CR:CD rats. This was based on reduced body weight, body weight gain, and food efficiency in male rats, and microcytic anemia, liver lesions, and kidney toxicity in both sexes seen at 10,000 ppm.

- According to the data from seven FIFRA guideline tests, pyraflufen-ethyl has no mutagenic or clastogenic properties and no effect on DNA repair in *in vitro* or *in vivo* bacterial or mammalian test systems. The seven studies are acceptable and satisfy the 1991 guideline requirements for mutagenicity. No further testing is required at this time.

- No appropriate structural analogues were located for comparison purposes.

- The CARC concluded that none of the data from four special non-guideline mouse liver studies provided evidence for a possible mode of action for the mouse liver tumors. These studies were designed to show effects on cytochrome P450s, liver enzymes associated with liver toxicity, and effects on metabolism.

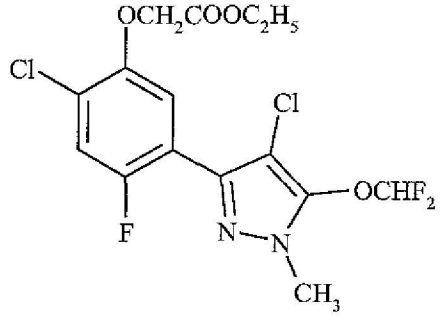
In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July 1999), the CARC classified pyraflufen-ethyl into the category **"Likely to be Carcinogenic to Humans"**. The Committee further recommended using a linear low-dose extrapolation approach for the quantification of human cancer risk based on male mouse combined liver tumors. The data did not support a mode of action.

I. INTRODUCTION

On August 14, 2002 the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of pyraflufen-ethyl. This was the initial assessment of this chemical for carcinogenicity by the HED CARC.

II. BACKGROUND INFORMATION

Pyraflufen-ethyl, (ET-751) (PC. Code: 030090), ethyl (ethyl 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetate), is a new active ingredient. It belongs to the phenylpyrazole class of chemicals called protox inhibitors. The chemical works by inhibiting the action of protoporphyrinogen IX oxidase, resulting in peroxidation of foliar cell membrane lipid in the presence of light, with subsequent cell membrane destruction and necrosis. The proposed use of pyraflufen-ethyl is to control post-emergent broadleaf weeds in cotton, potatoes and "non-crop areas" such as airports, nurseries, ornamental turf, roadsides and railroads. The structure is provided below:



Occupational exposure is expected. Non-food uses may be expected to result in residential/recreational exposure (postapplication-incidental oral (children), and in dermal exposure to adults and children. The primary routes of concern are dermal and inhalation, with time frames varying from days to many months.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity and Carcinogenicity Study in CR:CD Rats

References:

Patel, S. 1996. ET-751: Combined oncogenicity and toxicity study by dietary administration to CD rats for 104 weeks. Huntingdon Life Sciences, Ltd., Eye Suffolk IP23 7PX England, Report No. 96/NHH060/0448, T-5064, December 24, 1996. MRID 45282911. Unpublished.

A. Experimental Design

Pyraflufen-ethyl (Batch Nos. 3AM0019P and 4AM0021D, 97.6% a.i.; 5AM0026D, 97.7% a.i.) was administered in the feed to groups of 70 male and 70 female CR:CD rats at dietary concentrations of 0, 80, 400, 2000, and 10,000 ppm. These concentrations corresponded to 3.4, 17.2, 86.7, and 468.1 mg/kg/day for males and 0, 4.4, 21.8, 111.5, and 578.5 mg/kg/day for females. Twenty rats of each sex and dose level were sacrificed after about 52 weeks for interim evaluations, and the remaining animals were maintained on the treated or control diets until termination at 104 weeks.

B. Discussion of Tumor Data

The incidence of benign adrenal medullary pheochromocytoma was slightly increased in female rats at 2000 (2/37, 5%) and 10,000 (4/50, 8%) ppm vs. the control (1/50, 2%). Although all adrenals were examined microscopically in the controls and high dose groups and early deaths were examined in all dose groups, only adrenals with gross lesions in the 80, 400, and 2000 ppm dose groups were examined histologically. Some microscopic adrenal tumors could have been missed using this technique. Therefore, the only reliable statistic is the pairwise comparison of the control and high dose group, the results of which were not statistically significant (Table 1). The historical control incidence of benign pheochromocytoma in female CR:CD rats in studies completed since January 1993 (two studies) is 7.7% (mean) with a range from 5.3-10.0%. The historical control incidence of malignant pheochromocytoma in female CR:CD rats in studies completed since January 1993 (two studies) is 1.4% (mean) with a range from 0-2.7%. Malignant pheochromocytoma was not observed in female rats in this study.

Table 1. Pyraflufen-Ethyl – CR:CD Rat Study

Female Pheochromocytoma Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

Females

	Dose (ppm)				
	0	80[++]	400[++]	2000[++]	10000
benign pheochromocytoma incidence (%)	1/50 (2)	0/38 (0)	0/43 (0)	2/37 (5)	4/50 (8)
p-value					0.1811

[+]Number of tumor bearing animals/Number of animals examined microscopically.
++Only a select number of animals were examined microscopically as per the protocol for the 80, 400, and 2000 ppm dose groups. Statistics were reported for the 10,000 ppm group only.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

C. Non-neoplastic Lesions

The target organs are liver, kidney and hematopoietic system. Microscopic findings are summarized in Table 2. At 52 weeks, 10,000-ppm group male and female rats had significantly increased incidences of microscopic lesions in the liver compared with the controls. Periacinar hepatocyte hypertrophy, bile duct hyperplasia, and periacinar hepatocyte fatty vacuolation each were observed in 30% ($p<0.05$) of the 10,000-ppm group male rats compared with either 0% or 5% of controls. The incidence of focal inflammation with hepatocyte degeneration was only marginally increased (25%, vs 5% for controls, $p=0.09$). Bile duct hyperplasia also was observed in 30% ($p<0.01$) of 10,000-ppm group female rats compared with only 5% of the controls. No other microscopic lesions occurred with significantly increased incidences in male or female rats at 52 weeks.

Another target for pyraflufen-ethyl appears to be the kidney where multiple lesions were observed in both male and female rats fed the 10,000-ppm diet for up to 104 weeks. In male rats, the incidences of transitional cell hyperplasia, papillary necrosis/sloughing, and acute papillitis (40%, 32%, and 22%, respectively) were significantly ($p<0.01$) increased compared with those of controls (12%, 0%, and 0%, respectively). The incidences of papillary transitional cell hyperplasia, dilation/hyperplasia of the collecting ducts, acute pyelitis, dilated cortical tubules, cortical cysts, and hydronephrosis were marginally ($p=0.06$ or $p=0.01$) increased compared with those of controls (0% or 2%). The incidences of acute pyelitis and hydronephrosis were not significantly or marginally increased in 10,000-ppm group females compared with controls, but the incidence of cortical cysts

was marginally increased (8% vs 0% for controls). The incidences of the remaining kidney lesions in 10,000-ppm females, transitional cell hyperplasia (64%) papillary transitional cell hyperplasia (24%), papillary necrosis/sloughing (32%), acute papillitis (16%), dilation/hyperplasia of collecting ducts (24%), and dilated cortical tubules (20%) were significantly increased ($p<0.01$) compared with control incidences of 26% for transitional cell hyperplasia, 6% for papillary transitional hyperplasia, and 0% for the remaining lesions. In addition, the incidence of transitional cell hyperplasia of the urinary bladder was 12% ($p<0.01$) in 10,000-ppm group males compared with 0% for controls.

Bile duct hyperplasia was the only notable liver lesion in main study rats fed the test material for up to 104 weeks; the incidence was 80% in 10,000-ppm group males compared with 0% for the controls and 72% in 10,000-ppm group females compared with 18% for the controls. Females also had a significant ($p<0.05$) increase in the incidence of focal medullary hyperplasia in the adrenal medulla (14% compared with 2% for controls). Other microscopic lesions occurred with similar incidences in treated and control groups. No treatment-related lesions were observed in male or female rats fed the 80-, 400-, or 2000-ppm diets.

TABLE 2. Microscopic Findings in Rats Fed Pyraflufen-ethyl

Organ/Lesion	Dietary Concentration (ppm)				
	0	80	400	2000	10000
Males – 52 Weeks					
Liver [No. animals examined]	20	19	19	19	20
Periacinar hepatocyte hypertrophy	0	0	0	0	6*
Focal Inflammation/hepatocyte degen.	1	1	0	3	5†
Bile duct hyperplasia	1	0	0	0	6*
Periacinar hepatocyte fatty vacuolation	1	2	0	0	6*
Males – 104 Weeks					
Kidneys [No. animals examined]	50	50	50	50	50
Transitional cell hyperplasia	6	8	7	3	20**
Papillary transitional cell hyperplasia	1	1	3	2	6‡
Papillary necrosis/sloughing	0	1	1	0	16**
Acute papillitis	0	1	0	0	11**
Dilation/hyperplasia of collecting	0	0	0	0	4‡
ducts	1	1	1	1	6‡
Acute pyelitis	0	0	0	0	4‡
Dilated cortical tubules	1	1	0	0	5†
Cortical cyst(s)	1	0	1	0	5†
Hydronephrosis					
Liver [No. animals examined]	50	50	50	50	50
Bile duct hyperplasia	18	16	12	15	40**
Urinary Bladder [No. animals examined]	50	49	50	49	50
Transitional cell hyperplasia	0	3	2	1	6**
Females – 52 weeks					
Liver [No. animals examined]	19	20	19	19	20
Bile duct hyperplasia	1	0	0	0	6**
Females – 104 Weeks					
Adrenal Medulla [No. animals examined]	50	38	43	37	50
Focal medullary hyperplasia	1	3	3	0	7*
Kidneys [No. animals examined]	50	50	50	50	50
Transitional cell hyperplasia	13	7	5	7	32**
Papillary transitional cell hyperplasia	3	3	4	2	12**
Papillary necrosis/sloughing	0	0	0	1	16**
Acute papillitis	0	0	0	1	8**
Dilation/hyperplasia of collecting	0	0	0	2	12**
ducts	0	0	0	0	10**
Dilated cortical tubules	0	1	0	1	4‡
Cortical cyst(s)					
Liver [No. animals examined]	50	50	50	50	50
Bile duct hyperplasia	9	6	7	6	36**

Data taken from Tables 15B (pages 171-177) and 15H, (pages 214-230), MRID 45282911.
*p≤0.05, **p≤0.01, statistically significant, treated group compared with the control group.
†p=0.10; ‡p=0.06, statistics calculated by the reviewer using Fisher's exact test.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate, but not excessive, for assessment of carcinogenicity in both sexes. In the 10,000 ppm group, clinical signs of toxicity included perigenital urine staining in females, brown staining on the tail of females, and paper staining beneath the cages of both sexes. Mortality was unaffected by treatment. Survival at termination was 52, 50, 40, 34 and 42% for males, and 34, 30, 22, 34 and 36% for females, respectively. Females in the 10,000-ppm group weighed 3% to 7% less than controls during the first year of the study and weighed 9% less to 1% greater than controls during the second year of the study. Females in the 10,000-ppm group gained 3% less weight than controls during weeks 0-13, 16-17% less during weeks 13-26 and weeks 26-52, 9% ($p<0.01$) less during the first year of the study, 19% less (no statistics) during the second year, and 11% (N.S.) less during the entire study. Male rats fed the 10,000-ppm diet weighed 11-18% less than controls from week 2 to study termination and gained 20% less weight from weeks 0-13, 26% less from weeks 13-26, but only 3% less from weeks 26-52 and 52-104. The 10,000-ppm group male rats gained significantly less weight than controls (18%, $p<0.01$) during the first year of the study and 16% less ($p<0.05$) during the entire study. Food efficiency was 14% lower in males in the 10,000 ppm group over the first 14 weeks of treatment when compared to controls. Male rats consumed 14-18% more water during weeks 12, 26, and 52 and 38-42% more during weeks 78 and 104; female rats consumed 12-19% more at weeks 12, 26, 52 and 78 and 40% more at week 99.

In 10,000-ppm group male and female rats, a mild microcytic anemia was observed. The hematocrit, hemoglobin, mean cell volume (MCV), and mean cell hemoglobin concentration (MCH) showed mild, but statistically significant ($p<0.01$) decreases of 5-11% at weeks 14, 27, and 52 compared with the controls. During the second year of the study, the decreases were slightly more severe ranging from 12-16% at weeks 79 and 103. In addition, increased percentages of 10,000-ppm group male rats had abnormal erythrocytes, primarily spherocytes, anisocytes, and poikilocytes throughout the study. In 10,000-ppm group females, hematocrit, hemoglobin, and MCV were significantly decreased at weeks 14 and 27 compared with controls but not at weeks 52, 79, or 100. The decreases were extremely small, and did not exceed 5%. Increased numbers of females in the 10,000 ppm group also had abnormal erythrocytes, particularly spherocytes, at weeks at weeks 14, 27, and 52.

Clinical chemistry changes in 10,000-ppm group rats included increased serum ALP (33-113%), ALT (91-440%), AST (38-105%), and OCT (108-290%) activities at almost all time points in males and increased AST (34-71%) and OCT (91-98%) activities in females during the first year. Increased urea in 10,000-ppm male rats at 26, 79, and 103 weeks (81-141%) may be related to the kidney lesions; however, 10,000-ppm females had more notable kidney lesions than the males, yet serum urea was not elevated. Increases in total protein, albumin, and globulin levels occurred at various times during the study in both sexes; the increases were small compared with controls and were not clearly dose- or time-related. Overall, the clinical chemistry changes in 10,000-ppm group male and female rats appear to be related to the microscopic lesions in the liver and kidney.

Male rats fed the 10,000-ppm diet had increased incidences of granular kidney, hydronephrosis, and abnormal shaped kidneys as well as distended urinary bladder and abnormal contents in the urinary bladder. In females fed the 10,000-ppm diet, the incidences of abnormal shaped kidneys was significantly increased compared with the control incidences. Microscopic lesions in the liver that appeared to be related to treatment with the test material included periacinar hepatocyte hypertrophy, periacinar hepatocyte vacuolation, focal inflammation/hepatocyte degeneration, and bile duct hyperplasia; the incidences of which were significantly or marginally increased in 10,000-ppm group males at week 52. The incidence of bile duct hyperplasia also was significantly increased in 10,000-ppm group females at week 52. Bile duct hyperplasia was the only liver lesion that had a significantly increased incidence in main study rats of each sex. Multiple lesions occurred in the kidney of 10,000-ppm group male and female rats and included hyperplasia of transitional cells, hyperplasia of papillary transitional cells, papillary necrosis, acute papillitis, acute pyelitis, dilatation/hyperplasia of collecting ducts, dilated cortical tubules, cortical cysts, and hydronephrosis in one or both sexes. In addition, 10,000-ppm group males had transitional hyperplasia in the urinary bladder. Hepatocyte injury was not evident upon light microscopic examination, but electron microscopy showed some evidence of hepatocytic mitochondrial abnormalities in the male and female rats, particularly at the 2000- and 10,000-ppm dose levels.

A 90-day feeding study was used as the basis for dose selection in the chronic/carcinogenicity study in rats. In a 90-day oral toxicity study (MRID45282903, 45282901, 45282902, 45282904) pyraflufen-ethyl [ET-751 (96.8% a.i., batch # 3AM0011N] was administered to 10 CD rats/sex/dose in the diet at concentrations of 0, 200, 1000, 5000, 15,000 ppm (equivalent to 0, 17.8, 85.6, 455.5 or 1489.4 mg/kg bw/day in males or 19.4, 95.4, 499.0, or 1502.9 mg/kg bw/day in females). An additional 5 rats of each sex were maintained on the 0, 5000 and 15,000 ppm diets for 90-days and then allowed to recover for 8 weeks. Signs of toxicity occurred at the high dose (15,000 ppm) to 4 of 15 males and 5 of 15 females within the first 12 days of treatment and included thin build, hunched posture, underactivity, abdominal distention, piloerection, irregular/fast breathing, pallor, and death. Death occurred within the first 12 days to three 15,000 ppm males. There were no treatment related signs observed after Week 8 in either sex. Although food consumption was lower in the 15,000 ppm males than the controls only during the first two weeks of treatment (79 and 92% of control), a lower food efficiency (77% of control) was evident throughout the study. In addition, the body weight and body weight gain of high-dose male rats was decreased. Hematocrits, hemoglobin concentration, mean cell volumes and mean cell hemoglobins were low after 12 weeks of treatment in animals in the 15,000 ppm groups (90-94% of controls) and were associated with anisocytosis and spherocytosis in some animals. Both sexes of the high dose animals also had increased total leucocytes (131-144% of control) due to increased neutrophils and lymphocytes.

Blood chemistry changes (expressed as percent of control) in the 15,000 ppm animals included low total protein (95%) and albumin (91%) in males and females (93% and 85%), high alkaline phosphatase (196%), alanine (469%) and aspartate aminotransferase activities (210%), high cholesterol (122%), low glucose levels (85%), low α-1 globulin (72%) and high β-globulin (124%)

concentrations in the males. Relative kidney and spleen weights of 15,000 ppm males were higher than controls (approximately 122%) and relative and absolute spleen weights were increased in the 15,000 ppm females (both 122%). Periacinar hepatocytic hypertrophy was observed in two males at the 5000 ppm level and in 7 males at the 15,000 ppm level. This was supported by the electron microscopy which indicated increased smooth endoplasmic reticulum in periacinar hepatocytes in addition to the appearance of electron dense vacuoles in lysosomes and mitochondria.

The lowest dose at which an adverse effect was seen after two weeks of treatment was 15,000 ppm based on clinical signs, death, and significantly reduced food efficiency and body weight in males with a NOAEL of 5000 ppm. In a 28-day range finding study the LOAEL was 20,000 ppm based on significant toxicity including death and effects on spleen, bone marrow and hematopoietic systems with a NOAEL of 2000 ppm. Based upon the results of the 3-month study, the doses of 80, 400, 2000 or 10,000 ppm were selected for the chronic toxicity/carcinogenicity study.

Based on the results from the chronic/carcinogenicity study in rats, the 90-day feeding study, and 28-day range-finding study, the high dose level of 10,000 ppm in the chronic/carcinogenicity study in rats is considered to be adequate.

2. Carcinogenicity Study in (SPF) ICR Crj:CD-1 Mice

References:

Kuwahara, M. (1996) ET-751: 78-week oral oncogenicity study in mice. The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan. Project identification no. IET93-0099, T-5060, December 4, 1996. MRID 45282913. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 45282913, 45282910, 45282914, 45282915) ET-751 (lot no. 4AM0021D, 97.6% a.i. and lot no. 4AM0023D, 98% a.i.)] was administered to 60 (SPF) ICR (Crj:CD-1) mice/sex/dose in the diet at dose levels of 0, 200, 1000, or 5000 ppm (equivalent to 0, 20.99, 109.7, and 546.8 mg/kg bw/day for males and 0, 19.58, 98.3, and 523.7 mg/kg bw/day for females) for 78 weeks. Ten mice/sex/group were removed for an interim kill after 13 weeks of treatment.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Pyraflufen-Ethyl in either male or female mice (Memo, L. Brunsman, 7/16/02, TXR No. 0050918)

Tumor Analysis

Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 5000 ppm dose group with the controls, for hepatocellular adenomas, and adenomas, carcinomas and/or hepatoblastomas combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for hepatocellular adenomas, and adenomas, carcinomas and/or hepatoblastomas combined, both at $p < 0.05$ (Table 3).

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 5000 ppm dose group with the controls, for hepatocellular adenomas, and adenomas and/or carcinomas combined, all at $p < 0.01$ (Table 5).

The statistical analyses of both sexes were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Tables 3 through 6 for tumor analysis results.

The historical incidence of hepatocellular adenoma in male ICR (Crj:CD-1) mice from 9/91- 3/95

at the testing laboratory is 28.6% (mean) with a range from 13.5-38.5%. The historical incidence of hepatocellular carcinoma in male ICR (Crj:CD-1) mice from 9/91- 3/95 at the testing laboratory is 6.9% (mean) with a range from 0-21.2%. The historical incidence of hepatocellular adenoma in female ICR (Crj:CD-1) mice from 9/91- 3/95 (10 studies) at the testing laboratory is 2% (mean) with a range from 0-6.5%. Hepatocellular carcinoma did not appear in any female historical control ICR (Crj:CD-1) mice from 9/91- 3/95 at the testing laboratory.

The incidence of vascular tumors (combined hemangiomas/hemangiosarcomas) was 2/43 (5%), 4/47 (9%), 4/38 (11%) and 5/45 (11%) in male mice in the control, 200, 1000 and 5000 ppm groups (Table 4), respectively, and 3/48 (6%), 1/46 (2%), 2/41 (5%) and 5/48 (10%), in females in the control, 200, 1000 and 5000 ppm (Table 6), respectively. The historical control incidence of hemangioma/hemangiosarcoma over this 4-year period prior to study and 1 year after completion of the study (10 studies) is 1.2 % in female (high incidence of 6%) liver.

Table 3. Pyraflufen-Ethyl – (SPF) ICR Crj:CD-1 Mouse Study

Male Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	200	1000	5000
Adenomas (%)	16/47 (34)	12/48 (25)	24/44 (55)	31[a]/47 (66)
p =	0.0001**	0.2294	0.0391*	0.0018**
Carcinomas (%)	1/47 (2)	1/48 (2)	2[b]/44 (5)	1/47 (2)
p =	0.5613	0.7474	0.4750	0.7527
Hepato-blastoma (%)	0/47 (0)	0/48 (0)	1[c]/44 (2)	1/47 (2)
p =	0.1830	1.0000	0.4835	0.5000
Combined (%)	17/47 (36)	12[d]/48 (25)	25[e]/44 (57)	33/47 (70)
p =	0.0000**	0.1688	0.0386*	0.0009**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 39.
[a]First adenoma observed at week 39, dose 5000 ppm.
[b]First carcinoma observed at week 67, dose 1000 ppm.
[c]First hepatoblastoma observed at week 73, dose 1000 ppm.
[d]One animal in the 200 ppm dose group had an adenoma and a carcinoma.
[e]Two animals in the 1000 ppm dose group had a combination of an adenoma, a carcinoma and/or a hepatoblastoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Pyraflufen-Ethyl – (SPF) ICR Crj:CD-1 Mouse Study

Male Vascular[v] Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	200	1000	5000
Hemangiomas (%)	1/43 (2)	3/47 (6)	3/38 (8)	4[a]/45 (9)
p =	0.1605	0.3427	0.2624	0.1947
Hemangio-sarcomas (%)	1/43 (2)	1[b]/47 (2)	1/38 (3)	2/45 (4)
p =	0.2654	0.7301	0.7213	0.5172
Combined (%)	2/43 (5)	4/47 (9)	4/38 (11)	5[c]/45 (11)
p =	0.2004	0.3819	0.2804	0.2361

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[v]Vascular tumors were observed in the kidney, liver, skin and spleen.
[a]First hemangioma observed at week 56, dose 5000 ppm.
[b]First hemangiosarcoma observed at week 67, dose 200 ppm.
[c]One animal in the 5000 ppm dose group had an hemangioma and an hemangiosarcoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Pyraflufen-Ethyl – (SPF) ICR Crj:CD-1 Mouse Study

Female Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	200	1000	5000
Adenomas (%)	1/48 (2)	0/46 (0)	1/41 (2)	16[a]/48 (33)
p =	0.0000**	0.5106	0.7120	0.0000**
Carcinomas (%)	0/48 (0)	0/46 (0)	0/41 (0)	1[b]/48 (2)
p =	0.2623	1.0000	1.0000	0.5000
Combined (%)	1/48 (2)	0/46 (0)	1/41 (2)	16[c]/48 (33)
p =	0.0000**	0.5106	0.7120	0.0000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 59, dose 5000 ppm.
[b]First carcinoma observed at week 79, dose 5000 ppm.
[c]One animal in the 5000 ppm dose group had an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Pyraflufen-Ethyl – (SPF) ICR Crj:CD-1 Mouse Study

Female Vascular[v] Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	200	1000	5000
Hemangiomas (%)	3/48 (6)	1[a]/46 (2)	2/41 (5)	4/48 (8)
p =	0.1796	0.3247	0.5751	0.5000
Hemangio-sarcomas (%)	0/48 (0)	0/46 (0)	0/41 (0)	1[b]/48 (2)
p =	1.0000	1.0000	1.0000	0.5000
Combined (%)	3/48 (6)	1/46 (2)	2/41 (5)	5/48 (10)
p =	0.0896	0.3247	0.5751	0.3572

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[v]Vascular tumors were observed only in the liver.
[a]First hemangioma observed at week 68, dose 200 ppm.
[b]First hemangiosarcoma observed at week 79, dose 5000 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions

The liver, and possibly the adrenal gland are the target tissues in mice. Microscopic findings are summarized in Table 7. Effects of treatment were first seen in the liver at 1000 and 5000 ppm in males and at 5000 ppm in females at the 13-week interim study. Centrilobular hepatocellular swelling, hepatocellular vacuolation and microgranuloma were seen in 90-100% ($p<0.01$) of high-dose males and females and in 60-90% of mid-dose males compared to 0-50% in the controls. An increased incidence of brown pigment deposition in Kupffer cells was also seen in high-dose males (not statistically significant) and females compared to the control group. Hepatocellular necrosis (focal) was increased (not statistically significant) in males in the high dose group at 13 weeks-(incidence: 2/10, 1/10, 3/10 and 5/10 -control to high dose).

After 78 weeks of treatment, significantly increased incidences of acidophilic foci, clear cell foci, and brown pigment deposition in Kupffer cells were seen in high-dose males and females and in mid-dose males compared to the respective control groups. The incidences of microgranulomas were significantly increased in high-dose males and females, but not at the mid-dose compared to the controls. The incidence of focal hepatocellular necrosis was increased in high-dose males, and the incidences of single cell necrosis were increased in mid- and high-dose females. Centrilobular hepatocellular swelling continued to be increased in both males and females in the 5000 ppm group and males in the 1000 ppm group; in addition, females in the 1000 ppm group were also affected at this time. There was a decreased incidence of centrilobular hepatocellular fatty changes in high-dose males compared to the control after 78 weeks of treatment. Amyloid deposition, which is often seen in various organs in aging mice was decreased in high-dose females, and in the liver, spleen, and thyroid the incidence of amyloid deposition was decreased significantly at the middle dose compared to the control group. Fatty cell degeneration significantly decreased in males in the 5000 ppm group.

The only finding that showed an increased incidence in an organ other than the liver was an increase in brown pigment deposition in the cortico-medullary junction of the adrenal gland in high-dose males and females. This lesion was seen in main study animals, but was not seen in animals at 13 weeks. There were no histologic correlates for the eye opacity observed macroscopically.

TABLE 7: Selected incidences of microscopic findings after 13 and 78 weeks of treatment with ET-751[a]

Organ, treatment weeks, finding	0 ppm	200 ppm	1000 ppm	5000 ppm
MALES				
Liver - 13 weeks				
focal hepatocellular necrosis	2/10	1/10	3/10	5/10
centrilobular hepatocellular swelling	0/10[b]	2/10	9/10**	10/10**
hepatocellular vacuolation	0/10	2/10	6/10**	10/10**
Kupffer cell brown pigment deposition	0/10	0/10	0/10	3/10
microgranuloma	5/10	3/10	6/10	10/10*
Liver - 78 weeks				
focal hepatocellular necrosis	2/50	3/50	7/50	12/50**
centrilobular hepatocellular swelling	1/50	1/50	11/50**	12/50**
acidophilic foci	7/50	6/50	21/50**	37/50**
clear cell foci	0/50	1/50	7/50**	26/50**
hepatocellular vacuolation	0/50	0/50	1/50	6/50*
Kupffer cell brown pigment deposition	1/50	3/50	19/50**	32/50**
microgranuloma	17/50	14/50	24/50	30/50**
Adrenal - 78 weeks				
brown pigment deposition, cortico-medullary junction	6/50	1/50	8/50	18/50**
FEMALES				
Liver - 13 weeks				
focal hepatocellular necrosis	0/10	0/10	0/10	2/10
centrilobular hepatocellular swelling	0/10	0/10	0/10	9/10**
hepatocellular vacuolation	0/10	0/10	0/10	9/10**
Kupffer cell brown pigment deposition	0/10	0/10	0/10	5/10**
microgranuloma	3/10	7/10	5/10	9/10**
Liver - 78 weeks				
centrilobular hepatocellular swelling	0/50	1/50	9/49**	38/50**
acidophilic foci	2/50	1/50	2/49	12/50**
clear cell foci	0/50	0/50	0/49	5/50**
hepatocellular vacuolation	0/50	0/50	3/49	6/50*
Kupffer cell brown pigment deposition	0/50	2/50	3/49	16/50**
microgranuloma	12/50	17/50	15/49	33/50**
single cell necrosis	0/50	1/50	5/49*	8/50**
Adrenal - 78 weeks				
brown pigment deposition, cortico-medullary junction	1/50	3/50	3/49	12/50**

[a] Data obtained from pages 141-184 in the study report, MRID 45282913.

[b] Number of mice with lesion/number of mice examined (10 mice at the interim sacrifice and 50 mice in the main study [animals taken at terminal sacrifice plus those killed *in extremis* or found dead]).

* Significantly different ($p<0.05$) from the control.

** Significantly different ($p<0.01$) from the control.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered adequate, but not excessive, for assessment of carcinogenicity in both sexes based on liver toxicity (centrilobular hepatocellular swelling in both sexes, brown pigment deposition in the Kupffer cells) and anemia, albeit slight. [It is noted that the highest dose level tested of 5000 ppm in the carcinogenicity study was equivalent to 546.8 mg/kg/day in males and 523.7 mg/kg/day in females, considerably less than the limit dose of 1000 mg/kg/day and less than 750 mg/kg/day, the dose calculated using standard conversion factors. These dietary intakes were verified by the reviewer.] Additional support derive from the results of the 28-day feeding study in which liver toxicity and anemia were observed at 3000 and 10,000 ppm.

In the mouse carcinogenicity study, there were no treatment-related effects on mortality or body weight. The mortality of males at study termination in the control, 200, 1000, and 5000 ppm groups were 40, 34, 64, and 38%, respectively. The respective mortalities of females were 26, 32, 33, and 30%. There were no significant treatment-related changes in body weights during the study. The body weight gains in high-dose males and females were 92% and 98% of the control group weight gains, respectively, after 13 weeks of treatment and 93% and 96% of the control group weight gains, respectively, after 78 weeks. Food consumption was slightly increased and food efficiency were slightly decreased in males in the 5000 ppm group.

At 13 weeks (the only time point sampled in the study), the hematocrit, hemoglobin concentration, and erythrocyte count in high-dose males were decreased by 13%, 12% ($p<0.01$), and 9% ($p<0.05$), respectively, compared to the control group. The hematocrit, hemoglobin concentration, and erythrocyte count were decreased by only 8%, 6%, and 5%, respectively, in high-dose females. These changes are indicative of a slight anemia occurring fairly early in the study, especially in males. Platelet counts were increased in high-dose males and females by 23% ($p<0.05$) and 49% ($p<0.01$), respectively, compared to the controls; and the white cell count was decreased by 49% ($p<0.01$) in high-dose males.

The relative (to body weight) liver weight in high-dose males (22%) and the absolute (28%) and relative (26%) liver weights in high-dose females were significantly increased compared to the control groups after 13 weeks of treatment. After 78 weeks of treatment, both the absolute and relative liver weights were increased in high-dose males (73% and 61%, respectively) and the absolute liver weight (19%) was increased in high-dose females. The absolute liver weight was also increased in mid-dose males (58%) after 78 weeks of treatment. The liver weight changes can be correlated with a number of microscopic lesions seen during histopathology. At 13 weeks, centrilobular hepatocellular swelling, hepatocellular vacuolation and microgranuloma were seen in 90-100% ($p<0.01$) of high-dose males and females and in 60-90% of mid-dose males compared to 0-50% in the controls. An increased incidence of brown pigment deposition in Kupffer cells was also seen in high-dose males (not statistically significant) and females compared to the control group. Hepatocellular necrosis (focal) was increased (not statistically significant) in males in the high dose group at 13 weeks- (incidence: 2/10, 1/10, 3/10 and 5/10 -control to high dose).

After 78 weeks of treatment, significantly increased incidences of acidophilic foci, clear cell foci, and brown pigment deposition in Kupffer cells were seen in high-dose males and females and in mid-dose males compared to the respective control groups. The incidences of microgranulomas were significantly increased in high-dose males and females. The incidence of focal hepatocellular necrosis was increased in high-dose males, and the incidences of single cell necrosis were increased in mid- and high-dose females. Centrilobular hepatocellular swelling continued to be increased in both males and females in the 5000 ppm group and males in the 1000 ppm group; in addition, females in the 1000 ppm group were also affected at this time. There was a decreased incidence of centrilobular hepatocellular fatty changes in high-dose males compared to the control after 78 weeks of treatment. The only finding that showed an increased incidence in an organ other than the liver was an increase in brown pigment deposition in the cortico-medullary junction of the adrenal gland in high-dose males and females. This lesion was seen in main study animals, but was not seen in animals at 13 weeks.

Based on liver toxicity (centrilobular hepatocellular swelling in both sexes, brown pigment deposition in the Kupffer cells and acidophilic and clear cell foci in males, and single cell necrosis in females) the NOAEL was determined to be 200 ppm in the diet (20.99 mg/kg/day for males 19.58 mg/kg/day for females).

In the 28-day study at doses of 3000, 10000 and 30000 ppm, all animals died at the high dose. At 10000 ppm, there were significant decreases in hematology parameters in males (19-26%) and females (10-12%), significant increases in ALT, AST and bilirubin, and significantly increased liver and spleen weights in males (32-33% and 34-37% for liver and spleen, respectively) and females (32% and 10% for liver and spleen, respectively). At 3000 ppm, the only effects were decreases in hematology parameters (12% and 5-9% in males and females) and liver macroscopic observations.

It was the consensus of the committee members that the carcinogenicity of pyraflufen-ethyl was adequately tested in mice. However, it is noted that a few CARC members felt the doses could have been higher (i.e., the carcinogenicity study could have been conducted at the limit dose of 7000 ppm), especially in females, since there was no effect on mortality or body weight in males or females in the carcinogenicity study. In addition, the hematological effects observed in females were slight. The liver effects were non-specific in females and there were no pre-neoplastic lesions apparent. In the 28-day feeding study in mice, all animals died at 30,000 ppm. At 10,000 ppm, there were significant effects on hematology and clinical chemistry parameters. Liver and spleen weights were also decreased in both sexes. At 3000 ppm, there were slight decreases in hematological parameters as well as macroscopic liver changes.

IV. TOXICOLOGY

1. Metabolism

Four studies were conducted to examine the metabolism and disposition of ET-751 (pyraflufen-ethyl) in male and female Sprague-Dawley rats following a single 5 or 500 mg/kg oral dose, or a 14-day repeated dose (5 mg/kg/day). Reported in MRID 45282932, 45282933, and 45282934, groups of male and female rats were subjected to the dosing regimens above using [pyrazole-5-^{14}C] ET-751 (lot no. H-251-30; >98% radio chemical purity; >99% chemical purity) or [phenyl-U-^{14}C]-ET 751 (lot no. H-251-48, >99% radio chemical purity) and nonlabeled test article (lot no. 5AM0027P, 99.4% chemical purity). Excretion, tissue distribution, pharmacokinetic, and metabolite profiles were determined. In MRID 45282935, biliary excretion and metabolite profiles were assessed in male rats given a single 5 mg/kg dose of [pyrazole-5-^{14}C] ET-751 (lot no. H-251-30; >98% radio chemical purity; >99% chemical purity).

There were no biologically significant treatment-related effects noted during the course of the study. ET-751 was readily absorbed and excreted within 96 hours following a single or repeated oral dose of 5 mg/kg (plasma $t_{1/2}$ of 3-3.5 hrs). However, at a dose of 500 mg/kg, absorption was saturated as indicated by C_{max} values which did not reflect the 100-fold dose differential (2.7-2.8 µg eq/g for the low-dose group and 100-107 µg eq-hr/g for the high-dose group). Following single or multiple oral low doses (5 mg/kg) of ET-751, urinary excretion accounted for 27-33% of the administered radioactivity suggesting that a multiple exposure regimen did not affect the absorption/excretion processes. Urinary excretion was reduced to only 5-7% following a single 500 mg/kg dose. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups. Analysis of biliary excretion following a single 5 mg/kg dose showed that ~36% of the administered dose appeared in the bile. Based upon the excretion data, total bioavailability of a low dose was approximately 56%. Biliary excretion data were not available for a high-dose group which prevented a definitive assessment of bioavailability. Excretory patterns did not exhibit gender-related variability. However, plasma and blood clearance was more rapid in females than in males as shown by plasma/blood radioactivity time-course and the greater AUC values for males (32.3 vs 18.4 µg eq-hr/g for the low-dose group and 2738 vs 1401 µg eq-hr/g for the high-dose group). Radioactivity concentrations indicated tissue concentrations at or near detection limits (generally <0.01 µg eq/g and never exceeding 0.02 µg eq/g) at 96 hrs postdose for any tissues. Therefore, neither ET-751 nor its metabolites appear to undergo significant sequestration. Tissue burden data following compound administration did not suggest a specific target beyond those tissues, namely liver and kidney, which are associated with absorption and elimination of orally administered xenobiotics.

Both urinary and fecal metabolites were quantified by HPLC and most were identified using HPLC and TLC in conjunction with known standards. The metabolites identified (primarily hydrolysis and subsequent demethylation products) were consistent with Phase I processes. The major metabolic pathway appears to be a sequential hydrolysis and demethylation of the parent compound

to metabolites E-1 and E-9 both of which are quantitatively prominent components detected in the urine and feces from all treatment groups. Extraction efficiencies appeared to be excellent and most components in all of the matrices examined (urine, feces, plasma, and bile) were adequately quantified and characterized. The available data, based upon studies using both the pyrazole-5 and the uniformly labeled phenyl ring, affirmed the metabolism pathway proposed by the investigators.

2. Mutagenicity

According to the data from seven FIFRA guideline tests, pyraflufen-ethyl has no mutagenic or clastogenic properties and no effect on DNA repair in *in vitro* or *in vivo* bacterial or mammalian test systems. The seven studies are acceptable and satisfy the 1991 guideline requirements for mutagenicity. No further testing is required at this time.

(i) In a reverse gene mutation assay in bacteria (MRID 45282924), strains TA98, TA100, TA1535, TA1537 and TA1538 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to ET-751 (Lot No. 4AM0021D, purity 97.6% a.i.) in DMSO at concentrations of 156.3, 312.5, 625, 1250, 2500 or 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix) in two independent assays. The S9-fraction was obtained from Aroclor 1254 induced male CD rat liver. ET-751 was tested up to a limit concentration of 5000 µg/plate based on the absence of cytotoxicity in a preliminary cytotoxicity test. **There was no evidence of induced mutant colonies over background.**

(ii) In a mammalian cell gene mutation assay the TK locus (MRID 45327623) (MRID 45327623), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to ET-751 (Lot No. 4AM0021D, 97.0% a.i.) in dimethylsulfoxide (DMSO) at concentrations of 0, 10, 20, 30, 40 or 50 µg/mL for 4 hours in the absence of mammalian metabolic activation (S9-mix) and at concentrations of 0, 150, 200, 250, 300 or 350 µg/mL for 4 hours with S9-mix. Two independent assays were conducted. **There was no evidence of induced mutant colonies over background up to cytotoxic concentrations (50 µg/ml -S9; 350 µg/ml +S9).**

(iii) In mammalian cell gene mutation assays at the TK locus (MRID 45282928), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to ET-751 (Lot No. 4AM0021D, 97.6% a.i.) in dimethylsulfoxide (DMSO) at concentrations of 0, 10, 20, 40, 60 or 80 µg/mL for 4 hours in the absence of mammalian metabolic activation (S9-mix) and at concentrations of 0, 20, 40, 80, 120 or 160 µg/mL for 4 hours with S9-mix. A second assay was conducted at test material concentrations of 0, 20, 40, 60, 80 or 100 µg/mL without S9-mix and at concentrations of 0, 40, 80, 120, 160 or 200 µg/mL with S9-mix. Concentrations ≥ ≈160 µg/mL were insoluble; cytotoxicity was seen at 80 µg/mL -S9 and 160 µg/mL +S9. **There was no increase in the number of mutant colonies over background in the absence of S9-mix but a non-reproducible dose-related increase in the number of mutant colonies was seen in the presence of S9-mix.**

(iv) In a mammalian cell cytogenetics assay (Chromosome aberration) (MRID 45282927), human

primary lymphocyte cultures were exposed to ET-751 (Lot No. 4AM0021D, 97.6% a.i.) in DMSO at concentrations of 0, 650, 1300 or 2600 µg/mL for 19 or 43 hours without metabolic activation (S9-mix) or for three hours with S9-mix. A second assay was conducted at the same three concentrations but using a 19 hour sampling time only. Compound precipitation occurred at 2600 µg/mL +/-S9. **There was no evidence of chromosomal aberration induction over background.**

(v) In a differential killing/growth inhibition assay in bacteria (MRID 45282929), strains H17 (rec+) and M45 (rec-) of *B. subtilis* were exposed to ET-751 (Lot No. 4AM0021D, 98.1% a.i.) in DMSO on paper disks at concentrations of 0, 343.75, 687.5, 1375, 2750 or 5500 µg/disk in the presence and absence of metabolic activation (S9-mix). **There was no evidence of greater growth inhibition or cell killing in repair-defective strains compared to repair competent strains up to the limit of test material solubility.**

(vi) In an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes (MRID 45282930), ET-751 at doses of 600 and 2000 mg/kg body weight, was administered to five SPF outbred albino Hsd/Ola Sprague-Dawley male rats per test group by oral gavage (four of the five rats were used for hepatocyte culture). No signs of overt toxicity to the test animals or cytotoxic effects to the target cells were seen up to the limit dose (2000 mg/kg). **The mean net nuclear grain count was below zero for both doses at both treatment times indicating no induction of UDS as tested in this study.**

(vii) In a CD-1 mouse bone marrow micronucleus assay (MRID 45282931), five mice/sex/dose/harvest time were treated via oral gavage with ET-751 (97.6% a.i., batch # A4M0021D) in corn oil using the following exposure/sacrifice scenarios: a single dose at 0, 1250, 2500 or 5000 mg/kg with sacrifice at 24 hours; a single dose of 5000 mg/kg with sacrifice at 48 and 72 hours; and two doses of 2500 or 5000 mg/kg separated by 24 hours with sacrifice 24 hours after the second dose. Bone marrow cells were harvested immediately following sacrifice. ET-751 was tested to the limit dose of 5000 mg/kg body weight. Signs of compound toxicity were limited to piloerection, hunched posture in one female, and piloerection and hunched posture in one male receiving 5000 mg/kg. No bone marrow cytotoxicity was seen at any dose. **There was no statistically significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose or treatment time.**

3. Structure-Activity Relationship

Data are not currently available on chemicals with similar structure to pyraflufen-ethyl.

4. Subchronic, and Chronic Toxicity

a) Subchronic Toxicity

Rat

MRIDs: 45282903, 45282901, 45282902, 45282904: In a 90-day oral toxicity study, pyraflufen-ethyl [ET-751] (96.8% a.i., batch # 3AM0011N] was administered to 10 CD rats/sex/dose in the diet at concentrations of 0, 200, 1000, 5000, 15,000 ppm (equivalent to 0, 17.8, 85.6, 455.5 or 1489.4 mg/kg bw/day in males or 19.4, 95.4, 499.0, or 1502.9 mg/kg bw/day in females). An additional 5 rats of each sex were maintained on the 0, 5000 and 15,000 ppm diets for 90-days and then allowed to recover for 8 weeks.

Signs of toxicity occurred at the high dose (15,000 ppm) to 4 of 15 males and 5 of 15 females within the first 12 days of treatment and included thin build, hunched posture, underactivity, abdominal distention, piloerection, irregular/fast breathing, pallor, and death. Death occurred within the first 12 days to three 15,000 ppm males. There were no treatment related signs observed after Week 8 in either sex. Although food consumption was lower in the 15,000 ppm males than the controls only during the first two weeks of treatment (79 and 92% of control), a lower food efficiency (77% of control) was evident throughout the study. In addition, the body weight and body weight gain of high-dose male rats was decreased. Hematocrits, hemoglobin concentration, mean cell volumes and mean cell hemoglobins were low after 12 weeks of treatment in animals in the 15,000 ppm groups (90-94% of controls) and were associated with anisocytosis and spherocytosis in some animals. Both sexes of the high dose animals also had increased total leucocytes (131-144%) of control) due to increased neutrophils and lymphocytes.

Blood chemistry changes (expressed as percent of control) in the 15,000 ppm animals included low total protein (95%) and albumin (91%) in males and females (93% and 85%), high alkaline phosphatase (196%), alanine (469%) and aspartate aminotransferase activities (210%), high cholesterol (122%), low glucose levels (85%), low α-1 globulin (72%) and high β-globulin (124%) concentrations in the males. Relative kidney and spleen weights of 15,000 ppm males were higher than controls (approximately 122%) and relative and absolute spleen weights were increased in the 15,000 ppm females (both 122%). Periacinar hepatocytic hypertrophy was observed in two males at the 5000 ppm level and in 7 males at the 15,000 ppm level. This was supported by the electron microscopy which indicated increased smooth endoplasmic reticulum in periacinar hepatocytes in addition to the appearance of electron dense vacuoles in lysosomes and mitochondria.

The lowest dose at which an adverse effect was seen after two weeks of treatment was 15,000 ppm based on clinical signs, death, and significantly reduced food efficiency and body weight in males with a NOAEL of 5000 ppm. In the 28-day range finding study, the lowest dose at which an adverse effect was seen was 20,000 ppm based on clinical signs, death, and significantly reduced food efficiency and body weight in males and females; the NOAEL was 2000 ppm. After 28-days

in the range finding study the LOAEL was 20,000 ppm based on significant toxicity including death and effects on spleen, bone marrow and hematopoietic systems with a NOAEL of 2000 ppm.

The pyraflufen-ethyl LOAEL for the 90-day study was 15,000 ppm or 1489-1503 mg/kg/day, based on significant toxicity including clinical signs, death, effects on erythrocytes, alternations in clinical chemistry tests for liver function, and splenomegaly. The NOAEL was 5000 ppm or 456-499 mg/kg/day.

Mouse

MRID 45282910 : In a 28-day oral toxicity study ET-751[(99.8% a.i., lot # 2AM0005P] was administered to 6 (SPF) ICR (Crj:CD-1) mice/sex/dose in the diet at dose levels of 0, 3000, 10,000, or 30,000 ppm (equivalent to 0, 441.8 or 1414 mg/kg/day for males; 0, 491.9 or 1682 mg/kg/day for females). Chemical intake was not calculated for the 30,000 ppm concentration because of 100% mortality within the first week of treatment.

Clinical signs including decreased motor activity, distended abdomen, and wet or soiled fur were seen in animals at 30,000 ppm, and all animals at this dose died or were killed *in extremis* within the first week of treatment. The urinary bladders of several mice were distended with urine, the stomachs of all the high-dose mice were found to be distended with food, and the cecum contents were hardened. There were no clinical signs or treatment-related mortality seen at the lower doses. Mean body weights of males were decreased by about 2% at 3000 ppm and by 2-4% at 10,000 ppm after the first week of treatment. Average food consumption was also decreased by about 4% in the 10,000 ppm group, but was not decreased at 3000 ppm compared to the control group. Body weights and food consumption were not affected by treatment in females. Urinalysis done during the 4th week of treatment showed a treatment-related decrease in urine pH in both sexes compared to the control groups.

Hematological changes after 4 weeks of treatment included statistically significant, dose-related decreases of about 12% at 3000 ppm and 19-26% at 10,000 ppm in hematocrit, hemoglobin concentration, and erythrocyte counts in males compared to the control. Females were less affected with decreases of 5-9% at 3000 ppm and 10% and 12% ($p<0.01$) at 10,000 ppm for hematocrit and hemoglobin concentration and 3% (NS) for erythrocyte count compared to the controls. The mean cell hemoglobin and volume were both significantly decreased by 8-10% and the platelet count was increased by 32-34% in both sexes at 10,000 ppm compared to the control groups.

Blood chemistry changes included significantly elevated alanine and aspartate aminotransferase activities, and total bilirubin in both sexes at 10,000 ppm compared to the controls. The alanine and aspartate aminotransferase activities were elevated in some males at 3000 ppm, but the means were not significantly different from the control group due to high standard deviations. Glucose and triglycerides were both significantly decreased in both sexes at 10,000 ppm and triglycerides were significantly decreased in males at 3000 ppm. Alkaline phosphatase activity, creatinine, and

calcium levels were increased in males at 10,000 ppm.

Absolute and relative (to body) spleen and liver weights of males at 10,000 ppm were significantly increased by 32-33% and, 34-37% for spleen and liver, respectively, compared to the control; and the absolute and relative liver and kidney weights were increased by about 32% and 10%, respectively, in females. In comparison with the control animals, at 10,000 ppm the livers of 3/6 males and females were enlarged, 2/6 males and 5/6 females had livers with accentuated lobular patterns, and 3/6 males and no females had livers that were dark in color. At 3000 ppm, 2/6 males and 3/6 females had livers with accentuated lobular patterns, and 1/6 females had an enlarged liver. One female had an enlarged spleen at 10,000 ppm.

Dog

MRIDs 45282905; 45282909: In a 90-day oral toxicity study (MRID 45282905; 45282909) Pyraflufen-ethyl (97.0% a.i., Batch No. 4AM0024D) was administered to four Beagle dogs/sex/dose in capsules at dose levels of 0, 40, 200, or 1000 mg/kg bw/day.

There were no compound related effects on mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and microscopic pathology. **No LOAEL for pyraflufen-ethyl was established in this study. The NOAEL is the limit dose of 1000 mg/kg bw/day, the maximum dose tested.**

c) Chronic Toxicity

Rat

MRIDs 45282911, 45282912: Details of the study design, including dosing regimens, and findings are covered above under section III-1.

Results: See Section III. 1. D.

Mouse

MRID 45282913: Details of the study design, including dosing regimens, and findings are covered above under section III-2

Results: See Section III. 2. D.

Dog

MRID 45302624: In a chronic oral toxicity study, ET-751 (pyraflufen-ethyl; 97.3 and 97.7% a.i., batch #s 5AM0025D [MRID 45282907] and 5AM0026D [MRID 45282908], respectively) was

administered to 4 beagle dogs/sex/dose in capsules at dose levels of 0, 40, 200, or 1000 mg/kg bw/day for 52 weeks.

Treatment with up to 1000 mg/kg/day did not adversely affect survival, clinical signs, body weights or body weight gains, food or water consumption, hematology or clinical chemistry parameters, urinalysis, ophthalmoscopic findings, absolute or relative organ weights, or gross and histopathological findings.

5. Mode of Action (MOA) Studies

The CARC concluded that none of the data from four special non-guideline mouse liver studies provided evidence for a possible mode of action for the mouse liver tumors. These studies were designed to show effects on cytochrome P450s, liver enzymes associated with liver toxicity, and effects on metabolism.

Executive summaries of the special studies submitted are presented below.

1. This special study (MRID 45327622) was conducted to determine if pyraflufen-ethyl induced lipid peroxidation, β-oxidation activity, catalase activity, and 8-hydroxydeoxyguanosine (8-OH-dG) production in mouse liver. Groups of five male ICR mice were fed diets containing pyraflufen-ethyl (97.5% a.i., Lot No. 4AM0021D) at concentrations 0, 200, 1000, 5000, or 10,000 ppm for 7 days. The mice were sacrificed and the liver was excised, homogenized in physiological saline, and processed for determination of the activities listed above.

Mice fed the 10,000-ppm diet lost weight but absolute and relative liver weights were not significantly affected. Body weights were not significantly affected at other doses, but absolute and relative liver weights were significantly increased by 39% and 46% at 5000 ppm. Lipid peroxide in liver homogenates was increased about threefold in the 5000- and 10,000-ppm group mice, β-oxidation activity in liver was increased about 4.7-fold, and catalase activity in liver was only 14% of the control level in 5000-ppm group mice. 8-OH-dG adducts were increased at 10,000 ppm but not at 5000 ppm. **The increase in β-oxidation and the decrease in catalase activity in the liver contributed to the increase in lipid peroxide, which coincided with the induction of hepatocellular necrosis in mice fed 5000 ppm of pyraflufen-ethyl in the diet (MRID 45282921).**

This study to determine if pyraflufen-ethyl induced lipid peroxidation, β-oxidation activity, catalase activity, or the production of 8-hydroxydeoxyguanosine is **Acceptable/Nonguideline** and satisfies the purpose for which it was conducted.

2. In a special study to determine if liver enlargement in mice fed ET-751 was caused by induction of drug metabolizing enzymes (MRID 45327620), groups of five ICR male mice were (1) administered a single gavage dose of ET-751 (97.5% a.i., Lot No. 4AM0021D) in corn oil at 0,

5000, or 10,000 mg/kg or (2) fed ET-751 in the diet at concentrations of 0, 200, 1000, or 5000 ppm for 28 days. Positive controls were fed 1200 ppm phenobarbital for 28 days. In an *in vitro* study, ET-751 or its metabolite, E-1 (Lot No. 5AM4403S), was incubated with liver microsomes from untreated mice to determine the effect on the activity of drug-metabolizing enzymes.

No induction of hepatic drug-metabolizing enzymes occurred in male mice administered a single dose of 5000 or 10,000 mg ET-751 /kg by gavage or fed up to 5000 ppm ET-751 in the diet for 28 days. Microsomal protein levels were not significantly affected by either method of treatment; cytochrome P-450 content was decreased to 67, 63, and 74% of control values 6, 24, and 48 hours, respectively, after dosing with 10,000 mg/kg and to 56% of the control value after feeding 5000 ppm for 28 days. Likewise activities of specific drug-metabolizing enzymes, ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-dealkylase (PROD), aminopyrine-*N*-demethylase, (AMND) aniline hydroxylase (AN-OH), and ethoxycoumarin *O*-deethylase (ECOD) were decreased after a single dose of ET-751. The decreases were dose-related in most instances and achieved statistical significance at one or both doses at a few time points after dosing. After feeding 5000 ppm of ET-751 for 28 days, EROD, PROD, AMND, AN-ON, and ECOD activities were decreased to 42, 51, 22, 82, and 51%, respectively, of the control levels and AMND activity was reduced to 64% after feeding 1000 ppm. In addition, the relative liver weight (% body weight) at 5000 ppm was increased by 22% ($p<0.01$) due to a small non-significant increase in absolute liver weight and a small non-significant decrease in body weight. Male mice fed phenobarbital at a dietary concentration of 1200 ppm had significant increases in absolute and relative liver weights and in each of the hepatic drug-metabolizing enzymes.

The *in vitro* studies showed that overall cytochrome P-450 content was not affected by incubation with ET-751 or ET-1 (10-1000 µg/mL). EROD was reduced to 59% and 72% of control after incubating with 100 µg/mL ET-751 or 1000 µg/mL E-1, respectively; 1000 µg/mL of ET-751 or E-1 caused 80% and 48% reductions, respectively, in AMND activity. None of the *in vitro* decreases achieved statistical significance.

In conclusion, **this study on hepatic drug-metabolizing enzymes showed that liver enlargement in mice fed ET-751 did not involve induction of hepatic drug-metabolizing enzymes; administration of ET-751 (up to 10,000 mg/kg) by a single gavage dose or feeding up to 5000 ppm) for 28 days resulted in inhibition of hepatic drug-metabolizing enzyme activities. The inhibition may involve a direct interaction of ET-751 or its metabolite, E-1, with some of the enzymes may be due to possible inhibition of heme synthesis resulting in diminished heme incorporation into cytochrome P-450.**

This study to determine the effect of ET-751 on hepatic drug-metabolizing enzymes is **Acceptable/Nonguideline** and satisfies the purpose for which it was conducted.

3. In a carcinogenicity study (MRID 45282913) ET-751 (lot no. 4AM0021D, 97.6% a.i. and lot no. 4AM0023D, 98% a.i.)] was administered to 60 (SPF) ICR (Crj:CD-1) mice/sex/dose in the diet at

dose levels of 0, 200, 1000, or 5000 ppm (equivalent to 0, 20.99, 109.7, and 546.8 mg/kg bw/day for males and 0, 19.58, 98.3, and 523.7 mg/kg bw/day for females) for 78 weeks. In this special study (MRID 45282916), the proliferative activity of hepatocytes from livers of 8 mice/sex/dose from MRID 45282913 was examined after 13 weeks of treatment and at 78 weeks. Proliferative activity was measured using immunohistochemical staining for proliferating cell nuclear antigen (PCNA) in liver sections and expressed as the percent of positively staining cells.

The group mean hepatocyte proliferation as measured by the percent of PCNA labeling cells increased in males at 1000 ppm by about 317% ($p<0.05$) and 1250% ($p<0.01$) of the control after 13 and 78 weeks, respectively. In the 1000 ppm female group, the increases were about 490% ($p<0.05$) and 780% (NS) of the controls at 13 and 78 weeks, respectively. The mean PCNA labeling cells in animals that received 5000 ppm in the diet were 475% and 1900% of the male control group and 1150% and 1810% of the female control group at 13 and 78 weeks, respectively (all $p<0.01$).

Liver toxicity in the carcinogenicity study (MRID 45282913) was indicated by increased absolute and/or relative (to body) weights in both sexes at 5000 ppm after 13 and 78 weeks of treatment and by increased incidences of gross and microscopic liver lesions seen at 1000 and 5000 ppm in both sexes compared to the controls. The increased hepatocyte proliferation as a result of treatment-related liver toxicity was suggested as the likely mechanism for the increased induction of hepatocellular adenomas in mid- and high-dose males and in high-dose females.

The LOAEL for ET-751 in mice is 109.7 mg/kg/day for males and 98.3 mg/kg/day for females, based on increased hepatocyte proliferation. The NOAEL is 20.99 mg/kg/day for males and 19.58 mg/kg/day for females.

This special study is **Acceptable/Non-guideline** for the determination of hepatocyte proliferative activity in mice treated with ET-751.

4. In a special study (MRID 45327621) to determine if there is an association between induction of hepatocellular necrosis and elevation of serum AST and ALT activities in mice fed ET-751, groups of 20 male ICR mice were fed ET-751 (98.6% a.i., Lot No. 4AM0021D) at dietary concentrations of 0, 3000, 5000, or 10,000 ppm for up to 4 weeks followed by a 2-week recovery period on basal diet without added test material. Five mice per group were sacrificed after treatment for 1, 2, and 4 weeks and after a 2-week recovery period.

No high-dose mice survived beyond day 9 of the treatment period. This group showed clinical signs suggestive of neurological effects, lost weight during the first of treatment, had elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (16-fold, $p<0.05$ and 2-fold, N.S., respectively), had gross liver lesions consisting of accentuated lobular pattern and whitish yellowish spots, and a very low incidence of microscopic liver lesions, including hepatocellular necrosis.

All mice in the low- and mid-dose groups survived to scheduled sacrifices and showed no clinical signs. The mid-dose group had significantly decreased body weights starting at 2 weeks and continuing to the end of the recovery period (-7 to -12%). Serum AST and ALT activities were significantly elevated 2.4- to 2.5-fold and 8.1- to 9.2-fold, respectively, after 2 and 4 weeks, and alkaline phosphatase activity was elevated 1.3- to 2.4-fold. Serum enzyme activities returned to the normal range during the recovery period. Other serum chemistry changes were transient and not considered related to liver injury. Postmortem examination of the liver in low- and mid-dose mice showed elevated absolute and relative weights that were not clearly dose-related, gross lesions consisting of an accentuated lobular pattern at both doses after 2 weeks, an accentuated lobular pattern and whitish or yellowish spots or mottling after 4 weeks, and a continued accentuated lobular pattern after the 2-week recovery period. Microscopic findings after treatment for 1 week consisted of hepatocellular necrosis at the mid-dose level and cellular inflammation, cell proliferation, clear cell foci, hepatocellular hypertrophy, and phagocytosis of red blood cells by Kupffer cells and hepatocytes at the low- and mid-dose level. The same findings were noted after 2 and 4 weeks in addition to hepatocellular necrosis in the low-dose group. A greenish yellow pigment was observed in the hepatocytes of low- and mid-dose mice after treatment for 2 and 4 weeks. At the end of the 2-week recovery period, some findings had undergone a reversal, notably hepatocellular necrosis at both doses and cell proliferation at the low-dose.

In conclusion, **feeding of ET-751 to mice at a dietary concentration of 10,000 ppm is lethal, whereas concentrations of 3000 and 5000 were not lethal, but increased the serum AST and ALT activities and induced liver toxicity manifested by a variety of lesions including hepatocellular necrosis and cell proliferation; neither hepatocellular necrosis or cell proliferation corresponded with the increases in serum AST or ALT activities.**

This study to determine if an association existed between the induction of hepatocellular necrosis and elevation of serum AST and ALT activities is **Acceptable/Nonguideline** and satisfies the purpose for which it was conducted.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that pyraflufen-ethyl showed evidence of carcinogenicity due to the following:

- Evidence of carcinogenicity was seen in the liver of both sexes of one species (mouse) **(i.e., benign liver tumors were seen in male and female (SPF) ICR (Crj:CD-1) mice).**

- Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 5000 ppm dose group with the controls, for hepatocellular adenomas, and combined adenomas, carcinomas and/or hepatoblastomas, all at $p< 0.01$. There were also significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for hepatocellular adenomas, and combined adenomas, carcinomas and/or hepatoblastomas, both at $p < 0.05$. The incidence of hepatocellular adenomas in males was 16/47, 12/48, 24/44, and 31/47 for the 0, 200, 1000, or 5000 ppm dose levels, respectively. The incidence of combined hepatocellular adenomas, carcinomas and/or hepatoblastomas was 17/47, 12/48, 25/44, 33/47 for the 0, 200, 1000, or 5000 ppm dose levels, respectively. The incidence of hepatocellular adenomas at 1000 ppm (55%) and 5000 ppm (66%) was outside the historical control range (mean: 28.6%; range:13.5-38.5%) for hepatocellular adenomas. The CARC considered the increase in hepatocellular adenomas and combined adenomas, carcinomas, and /or hepatoblastomas to be treatment-related in males. There was no treatment-related increase in hepatocellular carcinomas.

- Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 5000 ppm dose group with the controls, for hepatocellular adenomas, and combined adenomas and/or carcinomas, all at $p< 0.01$. The incidence of hepatocellular adenomas and combined adenomas and/or carcinomas in females was 1/48, 0/46, 1/41, 16/48 for the 0, 200, 1000, or 5000 ppm dose levels, respectively for both. The incidence of hepatocellular adenomas at 5000 ppm (33%) was outside the historical control range (mean: 2%; range: 0-6.5%) for hepatocellular adenomas. The CARC considered the increase in hepatocellular adenomas and combined adenomas and/or carcinomas to be treatment-related in females. There was no treatment-related increase in liver carcinomas.

- The highest dose tested of 5,000 ppm in both sexes was considered to be adequate to assess the carcinogenicity of pyraflufen-ethyl in ICR (Crj:CD-1) mice, but not excessive, in both sexes, based on liver toxicity (centrilobular hepatocellular swelling in both sexes, brown pigment deposition in the Kupffer cells) and anemia, albeit slight. Additional support derive from the results of the 28-day feeding study in which liver toxicity and anemia were observed at 3000 and 10,000 ppm.

- **There was no treatment-related increase in any tumors in male and female rats.**

- The highest dose tested of 10,000 ppm was considered to be adequate to assess the carcinogenicity of pyraflufen-ethyl in CR:CD rats, but not excessive, in both sexes, based on reduced body weight, weight gain and food efficiency in male rats, and microcytic anemia, liver lesions, and kidney toxicity in both sexes.

2. Mutagenicity

- According to the data from seven FIFRA guideline tests, pyraflufen-ethyl has no mutagenic or clastogenic properties and no effect on DNA repair in *in vitro* or *in vivo* bacterial or mammalian test systems. The seven studies are acceptable and satisfy the 1991 guideline requirements for mutagenicity. No further testing is required at this time.

3. Structure Activity

- No appropriate structural analogues were located for comparison purposes.

4. Mode of Action Studies

- The CARC concluded that none of the data from four special non-guideline mouse liver studies provided evidence for a possible mode of action for the mouse liver tumors. These studies were designed to show effects on cytochrome P450s, liver enzymes associated with liver toxicity, and effects on metabolism.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the *EPA Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the majority of the CARC voted to classify pyraflufen-ethyl as **"Likely to be Carcinogenic to Humans"** by the oral route. There was considerable discussion among the CARC about the merits of a "Suggestive", instead of a "Likely", classification. Some members thought that since only benign liver tumors were observed in only one species without accompanying liver hyperplasia (which is commonly seen as a precursor to tumors; however, generalized effects on liver cytotoxicity were seen in proliferation studies), and since pyraflufen-ethyl is not mutagenic, the classification of "Suggestive" was supported. However, in accordance with the *EPA Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the majority of the CARC voted to classify pyraflufen-ethyl as **"Likely to be Carcinogenic to Humans"** by the oral route based on the following weight-of-the-evidence considerations:

1. Hepatocellular adenomas were seen in both male and female mice.

2. The increased occurrence of liver tumors in both male and female mice was robust in

comparison to the historical controls.

3. The occurrence of liver tumors in male mice showed a strong dose-response relationship.

4. The majority of the CARC felt that dosing was adequate, but not excessive, in both sexes of mice. However, some CARC members felt that the mice could have tolerated higher doses at which a progression towards malignancy (i.e., the production of hepatocellular carcinomas) might have occurred.

5. The liver was the target organ in two species, mouse and rat.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended using a linear low-dose extrapolation approach for the quantification of human cancer risk based on the incidence of male mouse combined hepatocellular adenomas, carcinomas and/or hepatoblastomas. The data submitted by the registrant did not support a mode of action for the mouse liver tumors.

VIII. BIBLIOGRAPHY

MRID No.	CITATION
45282901	A. Broadmeadow (1994) ET-751: Preliminary toxicity study by dietary administration to CD rats for four weeks. Final Report. Pharmaco LSR Ltd., Eye, Suffolk IP23 7PX , England. Report 93/NHH057/0824, April 6, 1994. Unpublished
45282903	A. Broadmeadow (1994) ET-751: Toxicity study by dietary administration to CD rats for 13 weeks followed by an 8 week reversibility period. Final Report. Pharmaco LSR Ltd., Eye, Suffolk IP23 7PX, England. Report 93/NHH058/1093, September 20, 1994. Unpublished
45282904	A. Broadmeadow (1994) ET-751: Toxicity study by dietary administration to CD rats for 13 weeks followed by an 8 week reversibility period, First Supplement to Pharmaco LSR Report 93/NHH058/1093 Electron MICR (CD-1)oscopy Report. Pharmaco LSR Ltd., Eye, Suffolk IP23 7PX, England. Report 93/NHH058/1094, September 20, 1994. Unpublished
45282905	Broadmeadow, A. (1996) ET-751: Toxicity study by oral (capsule) administration to beagle dogs for 13 weeks. Huntingdon Life Sciences Ltd, Suffolk, England. Report No. 95/NHH071/0275, September 19, 1996. Unpublished
45282906	Broadmeadow, A. (1996) ET-751: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks. Huntingdon Life Sciences, Ltd., Eye Suffolk, England, IP23 7PX. Report number 96/NHH083/0883, December 19, 1996. Unpublished.
45282909	Broadmeadow, A. (1994) ET-751: Preliminary toxicity study by oral (capsule) administration to beagle dogs. Huntingdon Life Sciences Ltd, Suffolk, England. Report No. 94/NHH070/0668, November 4, 1994. Unpublished
45282910	Takahashi, K. (1994) ET-751: 78-week oncogenicity study in mice, 28-day dose range finding study. The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan. Project identification no. IET93-0099, T-5002, May 2, 1994. Unpublished.
452829011	Patel, S. 1996. ET-751: Combined oncogenicity and toxicity study by dietary administration to CD rats for 104 weeks. Huntingdon Life Sciences, Ltd., Eye Suffolk IP23 7PX England, Report No. 96/NHH060/0448, T-5064, December 24, 1996. Unpublished.

45282913 Kuwahara, M. (1996) ET-751: 78-week oral oncogenicity study in mice. The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan. Project identification no. IET93-0099, T-5060, December 4, 1996. Unpublished.

45282914 Kuwahara, M. (2000) Supplement to ET-751: 78-week oral oncogenicity study in mice. The Institute of Environmental Toxicology, 4321, Uchimoriya-machi, Mitsukaido-shi, Ibaraki, 303-0043, Japan. Project identification no. IET93-0100, T-5060, Supplement 1, October 24, 2000. Unpublished.

45282915 Fox, S.P. (1994) Certificate of purity for ET-751 technical. Pharmaco LSR Ltd., Eye Suffolk, IP23 7PX, England. Project identification T-5060, Addendum 1, Report 94/1332, December 29, 1994. Unpublished.

45282924 May, K. (1994) ET-751: Assessment of mutagenic potential in amino-acid auxotrophs of *Salmonella typhimurium* and *Escherichia coli* (the Ames test). Pharmaco LSR, Ltd., Eye, Suffolk, IP23 7PX, England. Laboratory LSR Schedule No.: NHH/072, Laboratory LSR Report No.: 94/NHH072/0379, August 17, 1994. Unpublished

45282927 Dance, C.A. (1994) ET-751: *In vitro* tests for induction of chromosome damage: cytogenetic study in cultured human peripheral lymphocytes. Pharmaco LSR Ltd., Eye Suffolk, IP23 7PX, England. Pharmaco LSR Schedule No.: NHH/073, Pharmaco LSR Report No.: 94/NHH073/0786, T-5008, October 10, 1994. Unpublished

45282928 Lloyd, J.M. (1994) ET-751: L5178Y TK$^{\pm}$ mouse lymphoma mutation assay using the methodology recommended by the O.E.C.D. (1984). Pharmaco LSR Ltd., Eye Suffolk, IP23 7PX, England. Pharmaco LSR Schedule No.: NHH/074, Report No.: 94/NHH074/0600, T-5005, September 15, 1994. Unpublished

45282929 Miyahana K. (1994) ET-751: DNA Repair Test (Rec-Assay) with *Bacillus subtilis*. Nihon Nohyaku Co., Ltd., Research Center, 345, Oyamada-cho, Kawachi-Nagano, Osaka, 586-0094, Japan. Project ID: ILSR-T94-053C, GA-08, 94-0093, T-5011. November 10, 1994. Unpublished.

45282930 Gant, R.A. and R. Proudlock (1998) Rat Liver DNA Repair (UDS) Test. Huntingdon Life Sciences Ltd., PO Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Project ID: NHH099/982777, T-5091, April 30, 1998. Unpublished.

45282931 Edwards, C.N. (1994) ET-751: Mouse micronucleus test. Pharmaco LSR Ltd., Eye Suffolk, IP23 7PX, England. Pharmaco LSR Schedule No.: NHH/075, Pharmaco

LSR Report No.: 94/NHH075/0658, T-5004, August 24, 1994. Unpublished

45282932 Motoba, K. Absorption, distribution, metabolism and excretion of [pyrazole-5-^{14}C] ET-751 following a single oral administration to male and female rats. Report No. LSRC-M96-002A (translated), Nihon Nohyaku Co., Ltd., Research Center, 345 Oyamadacho, Kawachinagano, Osaka 586-0094, Japan. 25 September 1996. Study No. GB-01, 95-0044. Unpublished.

45282933 Motoba, K. Metabolism and excretion of [phenyl-5-^{14}C] ET-751 following a single oral administration to male and female rats. Report No. LSRC-M96-056A (translated), Nihon Nohyaku Co., Ltd., Research Center, 345 Oyamadacho, Kawachinagano, Osaka 586-0094, Japan. 2 December 1996. Study No. GB-01, 96-0109. Unpublished.

45282934 Motoba, K. Absorption, distribution, metabolism and excretion of [pyrazole-5-^{14}C] ET-751 following repetitive oral dosing of non-radiolabeled test substance to rats. Report No. LSRC-M96-033A (translated), Nihon Nohyaku Co., Ltd., Research Center, 345 Oyamadacho, Kawachinagano, Osaka 586-0094, Japan. 10 October 1996. Study No. GB-01, 96-0008. Unpublished.

45282935 Motoba, K. Metabolism and excretion of [pyrazole-5-^{14}C] ET-751 into bile following a single oral administration to rats. Report No. LSRC-M96-017A (translated), Nihon Nohyaku Co., Ltd., Research Center, 345 Oyamadacho, Kawachinagano, Osaka 586-0094, Japan. 22 October 1996. Study No. GB-01, 96-0019. Unpublished.

45327623 Tanaka, N. (1998) Report on Mutation Assay of ET-751 Using L5178Y Mouse Lymphoma Cells. Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano, Kanagawa 257-8523, Japan. Project ID: HRI Report No. 10-426, T-5092, July 14, 1998. Unpublished

HED Memorandum Dated July 16, 2002: Pyraflufen-Ethyl Qualitative Risk Assessment Based On (SPF) ICR Crj:CD-1 Mouse Dietary Study: From Lori Brunsman to William Greear.

13544

056222

Chemical:	**Ethyl 2-chloro-5-[4-chloro-(5-difluorome**
PC Code:	**030090**
HED File Code	**21200 CARC**
Memo Date:	**10/08/2002**
File ID:	**00000000**
Accession Number:	**412-03-0019**

HED Records Reference Center
12/10/2002